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BRIEF COMMUNICATION

CALR mutation profile in Irish patients with myeloproliferative neoplasms

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Abstract
Insertion and/or deletion mutations of the *CALR* gene have recently been demonstrated to be the second most common driver mutations in the myeloproliferative neoplasms (MPNs) of essential thrombocythemia (ET) and primary myelofibrosis (PMF). Given the diagnostic and emerging prognostic significance of these mutations, in addition to the geographical heterogeneity reported, the incidence of *CALR* mutations was determined in an Irish cohort of patients with MPNs with a view to incorporate this analysis into a prospective screening program. A series of 202 patients with known or suspected ET and PMF were screened for the presence of *CALR* mutations. *CALR* mutations were detected in 58 patients. Type 1 and Type 1-like deletion mutations were the most common ($n = 40$) followed by Type 2 and Type 2-like insertion mutations ($n = 17$). The *CALR* mutation profile in Irish ET and PMF patients appears similar to that in other European populations. Establishment of this mutational profile allows the introduction of a rational, molecular diagnostic algorithm in cases of suspected ET and PMF that will improve clinical management.

Introduction

The classical Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs) comprise the clinically and pathologically related polycythemia vera (PV), essential

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thrombocythemia (ET), and primary myelofibrosis (PMF). These diseases are characterized by an over production of mature hematopoietic cells, extramedullary hematopoiesis, and clinically by a tendency for thrombosis, hemorrhage, and a potential to transform into acute leukemia. While almost all PV patients are characterized by the Exon 14 *JAK2* V617F and Exon 12 mutations, until recently, the only known driver mutations of ET and PMF were the *JAK2* V617F and *MPL* Exon 10 mutations present in up to two-thirds of patients. The landmark discovery of insertion and/or deletion (indel) *CALR* Exon 9 mutations in 60—80% of *JAK2* V617F- and *MPL* Exon 10-negative ET and PMF cases compels the inclusion of *CALR* mutation analysis into the molecular diagnostic algorithm for these MPNs. The two common *CALR* mutations are the Type 1 52-bp deletion (p.L367fs*46) and the Type 2 5-bp insertion (p.K385fs*47), which account for more than 85% of indels [1,2] with most other mutations being Type 1-like deletions or Type 2-like insertions. *CALR* mutations are thought to exert their function by perturbation of the intracellular Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway by activation of *MPL* [3]. Molecular analysis of these three driver mutation types is likely to be a major criterion in the next revision of the World Health Organization classification of hematopoietic tumors [4]. Aside from their diagnostic importance, it is becoming increasingly apparent that the different types of *CALR* mutations impart prognostic information about thrombotic potential and leukemic transformation [5—7]. Furthermore, some geographical heterogeneity exists in both the frequency and profile of *CALR* mutations in ET and PMF when considering patients from North America [8,9], Europe [1,2,10,11], Central and South America [12,13], India [14], and Far East Asia [15,16].

Given the importance of *CALR* mutational analysis in the diagnosis and prognosis of ET and PMF, the pattern of *CALR* mutations was investigated in a cohort of adult Irish patients with MPN. These data are likely to provide information about how to efficiently incorporate *CALR* mutation analysis into a prospective MPN molecular diagnostic service.

Materials and methods

A series of 202 confirmed and suspected MPN cases from two large Irish hematology centers were subjected to retrospective *CALR* mutational analysis. None of the patients had evidence of the *JAK2* V617F mutation by allele-specific polymerase chain reaction (PCR) [17]. *CALR* mutations were detected in archival genomic DNA from peripheral blood ($n = 190$) or bone marrow ($n = 12$) by fluorescent PCR followed by fragment length analysis. The CALR assay has been previously demonstrated to possess a detection sensitivity of ~1% mutant allele burden [18].

Results

CALR mutations were detected in 58 patients (30 female and 28 male) with a median age of 56 years (range, 25—86 years) in whom the diagnosis was ET ($n = 45$), PMF ($n = 10$), post-ET myelofibrosis ($n = 2$), and myelodysplastic/MPNs ($n = 1$). Type 1 *CALR* mutations were the most prevalent, ($n = 35$) followed by Type 2 mutations ($n = 16$; Table 1). Of the remaining seven cases, five had Type 1-like deletions, one had a Type 2-like insertion, and one previously described patient harbored two mutated clones [19]. The distribution of these mutations in the MPN subtypes is given in Table 1.

Discussion

The description of *CALR* Exon 9 mutations in ET and PMF patients has changed the molecular diagnostic algorithm for MPN as they are now considered the second most common type of driver mutations in ET and PMF cases. Provision of a confirmatory molecular diagnosis in a significantly higher number of patients will enhance risk stratification and allows for appropriate treatment: retrospective screening has demonstrated the efficacy of existing therapeutic modalities such as interferon-alpha and JAK inhibitors in *CALR*-mutated MPN patients [20,21].

Because of the diagnostic and emerging prognostic significance of these mutations, in addition to the geographical and ethnic variation previously reported, the incidence of *CALR* indels was investigated in a retrospective cohort of Irish MPN patients to determine the existing mutational spectrum. Given the near mutual exclusivity of the *JAK2* V617F and *CALR* mutations but acknowledging the rarity of these mutations co-existing in ET and a report of *CALR* mutations in *JAK2*-negative PV [22—24], this retrospective study was limited to known and suspected ET and PMF patients in whom the *JAK2* V617F was previously undetected. Although a number of methodological approaches exist to detect *CALR* mutations [25], the sensitivity of the fragment length analysis used in this study was deemed sufficient to detect clinically relevant mutations: *CALR* mutant allele burdens are usually much higher [26].

Table 1 Incidence of *CALR* Mutations in Irish MPN Patients

	Type 1	Type 1 like	Type 2	Type 2 like	Other
ET ($n = 45$)	26	5	12	1	1
PMF ($n = 10$)	8	0	2	0	0
Post-ET MF ($n = 2$)	1	0	1	0	0
MDS/MPN ($n = 1$)	0	0	1	0	0
Total	35 (60.3%)	5 (8.6%)	16 (27.6%)	1 (1.7%)	1 (1.7%)

Note: ET = essential thrombocythemia; MDS/MPN = myelodysplastic/myeloproliferative neoplasm; MF = myelofibrosis; PMF = primary myelofibrosis.

The *CALR* mutation profile and frequency in this cohort of Irish patients are broadly in line with that of other series from Europe and North America, with Type 1 and Type 1-like mutations being more prevalent than Type 2 and Type 2-like in both ET and PMF patients. Establishing this mutational profile allows considered incorporation of *CALR* mutation testing into a prospective MPN molecular diagnostic algorithm so as to optimize the number of patients who would benefit from such testing within existing laboratory resources. Given the previously described variability in requesting *JAK2* V617F analysis within this country [27], together with the knowledge of the near absence of *CALR* mutations in splanchnic, cerebral, and other cases of unprovoked venous thrombosis [28—30], limiting testing to those patients with suspected ET and PMF appears judicious.

In conclusion, the incidence and spectrum of *CALR* mutations in Irish MPN patients are similar to those of other European cohorts confirming these driver mutations as the second most frequently observed in ET and PMF. This knowledge allows the effective incorporation of *CALR* mutation analysis into a prospective testing algorithm, thus enabling an enhanced diagnosis and improved clinical management.

Conflicts of interest

The authors declare no conflicts of interest regarding the publication of this paper.

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